PATENT
1257-116P

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT: STELLA, et al.

SERIAL NO.: 09/131,385 GROUP: 1614

FILED: August 7, 1998 EXAMINER: C. Aulakh

FOR: WATER SOLUBLE PRODRUGS OF HINDERED ALCOHOLS

DECLARATION SUBMITTED UNDER 37 C.F.R. § 1.132

Honorable Commissioner
Of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Dr. Roger Rajewski of the Center for Drug Delivery Research, University of Kansas, Kansas, the United States of America, do hereby declare the following:

I have attached a copy of my curriculum vitae to this Declaration.

I am associate director of the Center for Drug Delivery Research and managed the laboratory in which the experiments described below were conducted.

I am familiar with the above referenced patent application, as well as the development, usages and properties of prodrug compounds.

I have read and understand the subject matter of the Office Action of April 30, 1999.

The following comments are offered in support of the patentability of the instant invention.

Title: Studies of the activity of Phosphopropofol and the propofol prodrug O-Phosphonooxymethylpropofol

In Vivo anaesthetic studies

Purpose: To determine the utility of O-phosphonooxymethylpropofol and phosphopropofol as anaesthesia inducing agents. O-Phosphonooxymethylpropofol is a phosphate ester of propofol containing a methylene linker between the phenolic oxygen of propofol and the phosphate ester. Phosphopropofol is contains a phosphate ester directly attached to the phenolic oxygen of propofol.

Procedure: Male balb/c mice in the weight range of 18-24 grams were allowed to settle in cages for a period of 30 minutes following transfer from the animal room. Prior to injections, each mouse was placed in a warming box (~36 °C) for about 15 minutes to ensure dilation of tail veins to facilitate intravenous administration of drug. Groups of ten mice were used at each dose tested. Injections were given iv into tail veins over a ten second period. During the period of sleep mice were placed on a warming pad to maintain body temperature. The mice were stimulated by gentle stroking of the abdomen to more accurately observe waking. Dose range was chosen which produces lethality at one end and no loss of righting reflex at the other. For each mouse, sleep time (loss of righting reflexes), waking time and coordination time were recorded. The dose required to abolish righting reflexes for a minimum period of 30 seconds in 50% of mice (the median hypnotic dose, HD50) was estimated. Mice were euthanized by CO_2 inhalation at the end of the study.

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Results and Conclusions: The HD50 for the prodrug

O-phosphonooxymethylpropofol was found to be 36.72 mg/kg (propofol equivalents). At a dose of 40 mg/kg eight out of ten mice had induced anaesthesia while at 45 mg/kg anaesthesia was induced in all ten mice. The maximum tolerated dose or the lethal dose using this prodrug was greater than 100 mg/kg (propofol equivalents). The HD50 for phosphopropofol could not be determined as none of the mice slept at a propofol equivalent dose of 36.72 or 70 mg/kg. Phosphopropofol thus was not effective in inducing anaesthesia in mice and did not function as a prodrug of propofol.

In Vivo toxicity studies

Purpose: To evaluate the gross toxicity of O-phosphonooxymethylpropofol and phosphopropofol in rats.

Procedure: O-Phosphonooxymethylpropofol was prepared for i.v. injection at a concentration of 68 mg/mL in 0.9% Sodium Chloride Injection, USP. This concentration is equivalent to 36 mg/mL of propofol. Phosphopropofol was prepared for i.v. injection at a concentration of 43 mg/mL in 0.9% Sodium Chloride Injection, USP. This concentration is equivalent to 30 mg/mL of propofol. The prodrug solutions were filtered through a 0.22 µm nylon membrane prior to administration.

The evaluation of the propofol prodrugs on rats was conducted with two male Harlen Sprague-Dawley rats for each prodrug. For O-phosphonooxymethylpropofol, the rats were given doses of 9 mg/kg (propofol equivalents) in the tail vein. For phosphopropofol, the rats were given doses of approximately 12 mg/kg in the tail vein.

Results: For O-Phosphonooxymethylpropofol, both rats became unsteady after a few minutes and were very calm. Based on visual observations, the rats fully recovered from the propofol prodrug injections. Blood removed from both rats

confirmed the presence of propofol through HPLC analysis. The rats did not display signs of discomfort due to the propofol prodrug. For phosphopropofol, both rats became very excited following the injections for approximately three minutes. The rats never became unsteady and appeared to be in a great deal of discomfort. Based on the results of these studies it was decided that phosphopropofol would not be administered to higher animal species (dogs, rabbits, etc.).

In Vitro studies

Purpose: To evaluate if O-phosphonooxymethylpropofol and phosphopropofol are acceptable substrates for alkaline phosphatase.

Procedure: To evaluate the in vitro conversion of propofol prodrugs (O-Phosphonooxymethylpropofol and Phosphopropofol) to propofol, the prodrugs were treated at 37°C with alkaline phosphatase in glycine buffer at pH 10. To compare the rate of conversion of the prodrugs to propofol, samples were taken at different time intervals and the amount of propofol at each time point was quantitated using an HPLC method.

Results: Propofol prodrugs (O-Phosphonooxymethylpropofol and Phosphopropofol) were both converted in vitro to propofol by alkaline phosphatase. However, the rate of conversion to propofol was found to be significantly lower with phosphopropofol as compared to O-Phosphonooxymethylpropofol.

The undersigned hereby declares that all statements made herein based upon knowledge are true, and that all statements made based upon information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED: 19AUG99

Dr. Roger Rajewski

Enclosures: As stated above

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope to: Commissioner of Patents and Trademarks, Washington D.C. 20231 on: 8-26-99
(Date of deposit)

BIRCH, STEWART, KOLASCH & BIRCH, LLP

(Signature)

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(Date of Signature)